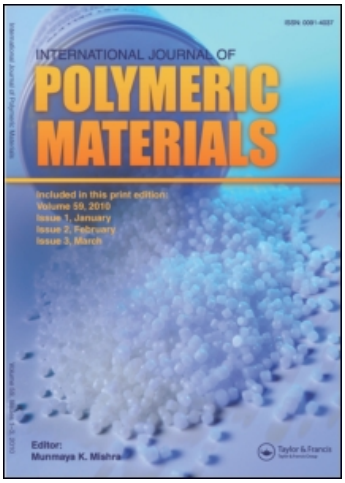

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Novel pH-sensitive Carriers Containing Naproxen Pendant Groups for Colon-Specific Drug Delivery

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The carboxyl group of naproxen was converted into a vinyl ester group by reacting naproxen with vinyl acetate in the presence of mercuric acetate as a catalyst. Cubane-1, 4-dicarboxylic acid (CDA) was covalently linked with 2-hydroxyethyl methacrylate (HEMA) as the crosslinking agent (CA). Methacrylic-type polymeric prodrugs were synthesized by free radical copolymerization of methacrylic acid, vinyl ester derivative of naproxen (VIN) and polyethylene glycol monomethacrylate (PEGMA) in the presence of a cubane crosslinking agent. In vitro release profiles were established separately in enzyme-free simulated gastric and intestinal fluids (SGF, pH 1 and SIF, pH 7.4).

Keywords cubane, hydrogel, naproxen, oral drug delivery, pH-sensitive, release

INTRODUCTION

Although oral delivery has become a widely accepted route for the administration of therapeutic drugs, the gastrointestinal tract presents several formidable barriers to drug delivery. Colonic drugs delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of

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proteins and therapeutic peptides. To achieve successful colonic delivery, a drug needs to be protected from absorption in the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs.

One strategy for targeting orally administered drugs to the colon includes covalent linkage between drug and pH-sensitive hydrogel in such a manner that upon oral administration the moiety remains intact in the stomach and small intestine. The chemical attachment of drugs to synthetic polymers is one means of increasing the duration of activity through slow release, or target-directing drugs in the body [1].

Poly(ethylene glycol) (PEG) is widely used in the drug delivery system (DDS) for many reasons, with one being their low toxicity to cells [2]. Another reason is that PEGs bind relatively little with proteins [3], thereby enabling the long chain of the PEGs to protect the proteins and peptides in the DDS that are used to target the cells from reacting with other sites in the body [4]. PEGs may also increase the chances of the DDS carriers to reach the desired cells, as the large molecular weight of the PEGs have been reported to increase the circulation time of the DDS carriers in the bloodstream [5].

Responsive hydrogel networks consisting of polymethacrylic acid (PMAA) and polyethylene glycol (PEG) are classic examples of pH-sensitive carriers that exhibit swelling transitions in response to changes in pH [6, 7]. PEG and PMAA may be associated to form hydrogen-bonded complexes under acidic conditions. A hydrogel containing a backbone of PMAA and grafts of PEG exhibits a relatively low degree of swelling under complex-promoting conditions (low pH when acid is protonated) and a high degree of swelling when the complex is broken (high pH when the acid is neutralized) [8]. Several hydrogel networks, including a PMAA backbone and PEG grafts, were synthesized and used to protect sensitive drugs from proteolytic degradation in the stomach and upper portion of the small intestine. In all cases, the drug was physically trapped in a polymer matrix, and drug release occurred via diffusion from the interconnected hydrogel matrix [9–11].

In this research, the synthesis of a pH-sensitive hydrogel network consisting of VIN, MAA, and polyethylene glycol monomethacrylate (PEGMA) is described. Complex-forming constituents of the hydrogel were covalently linked to each other and to the drug-linked monomer, and the swelling characteristics and drug-release properties of the hydrogel were studied in simulated gastric fluid (SGF, pH 1) and simulated intestinal fluid (SIF, pH 7.4).

EXPERIMENTAL

Materials

Cubane-1, 4-bis (methacryloyloxyethyl)carboxylate (CA) and poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) were prepared by the

methods described in the literature [12, 13], respectively. Poly(ethylene glycol) monomethyl ether (PEGME) was purchased from Aldrich (France) (Mn = 1000). Poly 2-hydroxyethyl methacrylate (HEMA), methacrylic acid (MAA) and dicyclohexylcarbodimide (DCC) were purchased from Merck Co., Ltd. (Germany). 4-dimethylaminopyridine (DMAP) and reagents were obtained from Fluka Co., Ltd. (Tokyo, Japan). Methacrylic acid was purified by distillation under vacuum. Initiator of 2,2′-azobisisobutyronitrile (AIBN) was purified by crystallization from methanol.

Instrumental Measurements

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker 400 AC spectrometer in $CDCl_3$. The IR spectra were recorded on a Shimadzu FTIR-408 spectrophotometer. The amount of released ibuprofen was determined by a Philips PU 8620 UV spectrophotometer at the maximum adsorption of the free drug in aqueous buffered solutions ($\lambda_{max} = 315$ nm) using a 1 cm quartz cell. Enzyme-free SGF (pH 1) or SIF (pH 7.4) was prepared according to the method described in the US Pharmacopeia [14].

Preparation of Naproxen Vinyl Ester (VIN)

The amount of 2.9 g (12.6 mmol) of naproxen and 0.3 g of mercuric acetate were dissolved in 30 ml of vinyl acetate and stirred for 30 min at room temperature. Then, 0.2 ml of concentrated sulfuric acid was added into the solution and refluxed for about 3 h. After this time, the solution was cooled to room temperature and 1.0 g of sodium acetate was added to quench the catalyst. The solution was filtered, concentrated and the crude product was then purified by silica gel column chromatography by eluting with petroleum ether/ethyl acetate (30:1, v/v) to give 2.5 g (85%) of VIN (Figure 1).

FTIR (KBr, cm^{-1}) 3050 (C−H aromatic and vinylic), 2890 (C−H aliphatic), 1750 (C=O ester), 1644, 1480 (C=C).

^{1}H NMR ($CDCl_3$, ppm) 1.64 (3H, d, J = 7.2 Hz, CH_3), 2.11 (1H, m, C_6H_4CH), 3.94 (3H, t, J = 10.6 Hz, CH_3O), 4.58 (1H, dd, J = 1.3 and 6.2 Hz, CH_2=C), 4.88 (1H, dd, J = 1.3 and 13.9 Hz, CH_2=C), 7.29 (1H, dd, J = 5.6 and 14.7 Hz, CH_2=CH), 7.38–7.15 (6H, m, ArH).

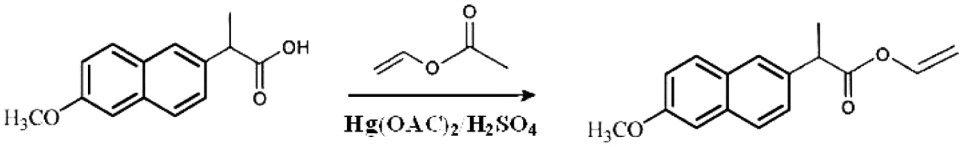


Figure 1: The synthesis route of vinyl ester type derivative of naproxen(VIN).

^{13}C NMR ($CDCl_3$, ppm) 18.8, 45.6, 55.7 (aliphatic carbons), 126.5 (1C, CH_2=CH–), 158.1 (1C, CH_2=CH–), 98.3, 106, 119.5, 127.7, 129.3, 129.7, 134.3, 135.2, 141.8 (aromatic carbons), 172.1 (1C, C=O).

Preparation of pH-Sensitive Hydrogels

Drug-linked copolymers were synthesized by copolymerization of MAA, PEGMA and VIN in a solution of methanol with a variable feed ratio as shown in Table 1. Terpolymerization was carried out in the presence of 2,2′-azobis isobutyronitrile (AIBN) as an initiator (0.01 molL-1) at 60–70°C in a thermostatic water bath. All experiments were carried out in Pyrex glass ampoules sealed off under vacuum. After the desired time (48 h) the precipitated network polymer-bonded drug was collected, washed with nonsolvent several times, dried under vacuum at room temperature and stored in desiccators until use (Scheme 1). IR (KBr): 3370-2550 (broadened, –COOH group), 1740, 1660, 1620, 1450, 1245, 1220 cm^{-1}.

Method of Hydrolysis

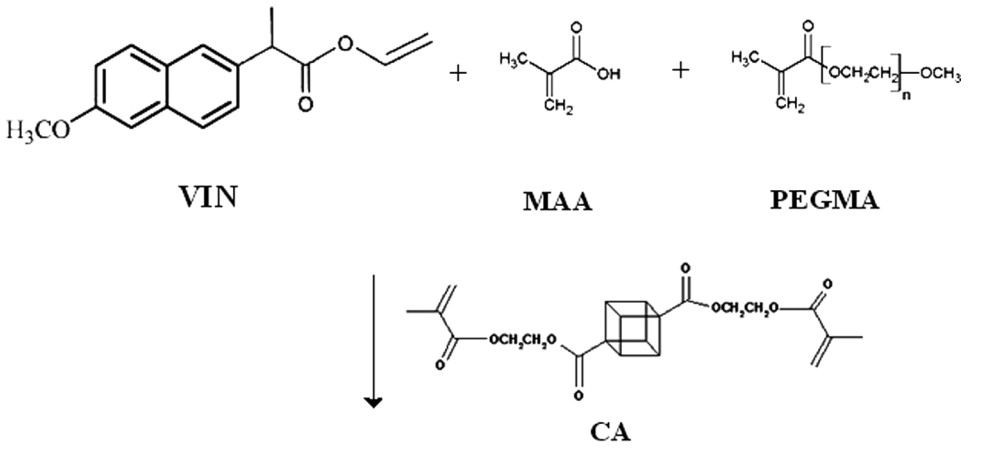
The polymer–drug conjugate (200 mg) was poured into 5 ml of aqueous buffered solution SGF (pH 1) or SIF (pH 7.4) at 37°C and the mixture was conducted into a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 25 ml of the same buffer solution maintained at 37°C. The external solution was continuously stirred and a 3-ml sample was removed at selected intervals and 3 ml of buffer was replaced. The quantity of released drug was analyzed by means of an UV spectrophotometer and determined from the calibration curve obtained previously under the same conditions.

Characterization of Hydrolysis Products

Fifty milligrams of polymer-drug adduct was dispersed in 20 mL of pH 8 buffered solution. The reaction mixture was maintained at 37°C. After 24 h

Table 1: Composition of copolymers

	Molar composition of monomers in the feed			
Polymers	**VIN**	**MAA**	**PEGMA^{1000}**	**CA**
P-1	1	3	1	5
P-2	1	3	2	5
P-3	1	3	1	10
P-4	1	3	2	10
P-5	1	5	1	5
P-6	1	5	2	5
P-7	1	5	1	10
P-8	1	5	2	10



Scheme 1: Preparation of network polydrug.

the hydrolysis solution was sampled and neutralized with 1 molL-1 hydrochloric acid and the solvent was evaporated in vacuo. The resulting crude product was treated with 10 mL of acetone and heated. The suspension was then filtered and the acetone solution was evaporated under reduced pressure. The residue was characterized by melting point measurement and IR spectroscopy and showed that the hydrolysis product is naproxen.

Measurement of Swelling Ratio

The hydrogels were characterized by the equilibrium swelling ratio. Dried copolymers were allowed to swell in 50 mL of enzyme-free SGF (pH 1) or SIF (pH 7.4) at 37°C. At a specific time, the hydrogel was removed from the swelling medium and the excess solution on the swollen sample was absorbed by gentle tamping between filter paper. The sample was weighed, and the procedure was repeated until a constant weight was achieved. The degree of swelling was calculated according the relation:

$$\mathrm{SW}\,(\%) = [(\mathrm{W_s} - \mathrm{W_d})/\mathrm{W_d}] \times 100$$

where, $\mathrm{W_s}$ and $\mathrm{W_d}$ represent the weight of swollen and dry samples, respectively. The swelling value of crosslinked polymers in pH 1 and pH 7.4 at 37°C are given in Table 2.

In situ Bioadhesivity Studies

Bioadhesivity testing was done by a novel in situ method as described by Ranga Rao and Buri [15]. A freshly cut 5–6 cm long piece of small intestine of

Table 2: Percent of swelling and percentage of particles adhered onto rat intestine

Polymers	Maximum constant swelling (%)		Percentage adherence
	pH 1	pH 7.4	
P-1	180	530	53
P-2	190	570	58
P-3	120	470	50
P-4	110	510	55
P-5	120	680	57
P-6	130	710	62
P-7	80	620	59
P-8	85	660	65

rat was obtained and cleaned by washing with isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of hydrogels were added evenly on the mucosal surface. The intestinal piece was maintained at 80% relative humidity for 30 min in a desiccator. The piece was taken out and phosphate buffer pH 6 was allowed to flow over the intestinal piece for about 2 min at a rate of 20 ml/min. The perfusate was collected and dried to get the particles not adhered. The percent of bioadhesion was estimated by the ratio of the amount applied to adhere hydrogels. The values are given in Table 2.

RESULTS AND DISCUSSION

Yang et al. [16] and Cai et al. [17] have already reported a method for the conversion of carboxylic acids to the related vinyl ester by using vinyl acetate as an acylating agent. In this present work, naproxen reacted with vinyl acetate in the presence of mercuric acetate as a catalyst, and the related vinyl ester (VIN), was collected in high yield after purification by column chromatography. The resultant FTIR and ^{1}HNMR spectra confirmed the structure of VIN and its purity. The related ^{1}H and ^{13}C NMR spectra of VIN are shown in Figures 2 and 3, respectively.

As shown in Table 2, an increase in the content of MAA in the feed monomer mixtures resulted in less swelling in SGF but greater swelling in SIF. The hydrogen-bonding and electrostatic interactions increased with MAA content in the copolymer networks. Because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH.

All the matrices with the presence of PEG and increase in the content of MAA had shown increased bioadhesivity (Table 2). The binding of those with sialic acid residues makes prolonged contact of the drug with the epithelium,

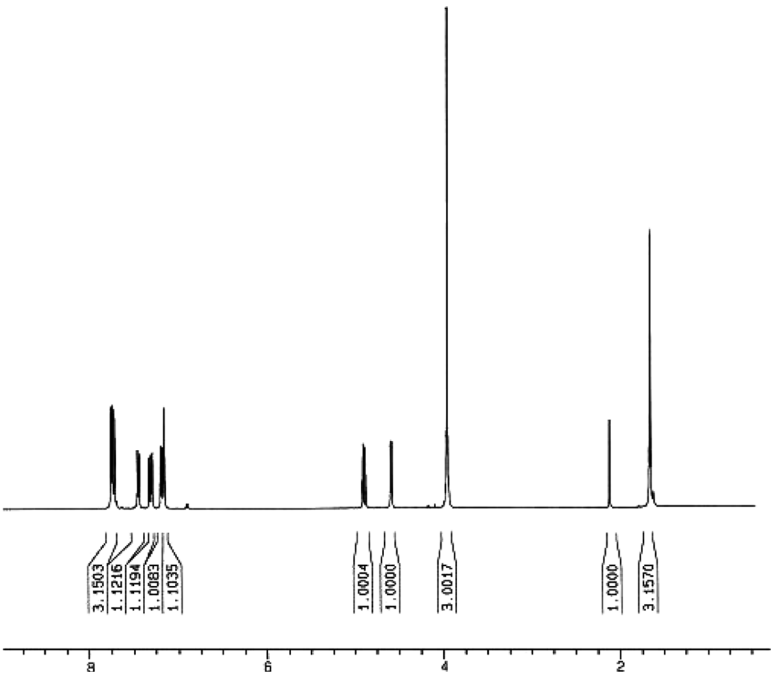


Figure 2: ^{1}H NMR spectrum of VIN in $CDCl_3$.

also it was assumed that the opening of the intercellular junctions by PEG could lead to the enhancement of insulin absorption across the mucosa.

Drug Release by Hydrolysis of Polymeric Prodrugs

It has been widely demonstrated that the side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the drug

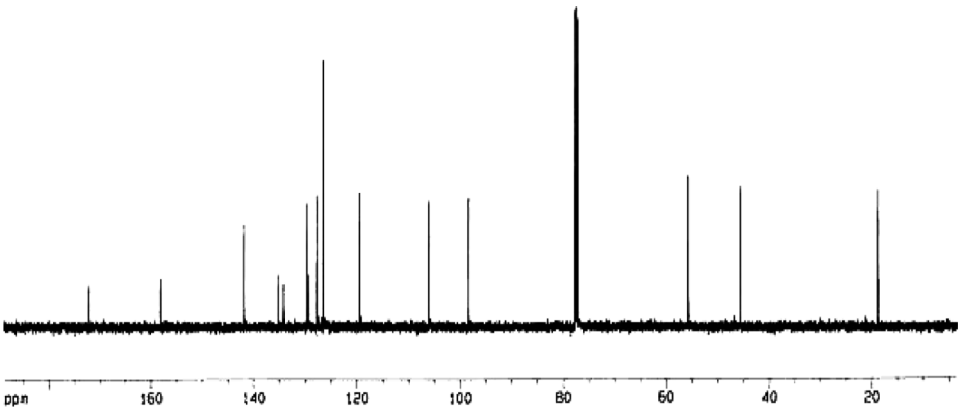


Figure 3: ^{13}C NMR spectrum of VIN in $CDCl_3$.

polymer chemical bonds, the structure of the polymer and the surrounding condition. The hydrolysis of a linkage is also dependent on its distance from the polymer backbone.

It appears that the degree of hydrolysis of network polymers depends on the amount of the MAA units in copolymer and the reticulated degree of crosslinking. With increased crosslinking and an increase in the reticulated degree of the polymer, diffusion of the hydrolyzing agents in the network's polymer is reduced and the hydrolysis rate is slower.

Figure 4 shows the release profiles of hydrogels at 37°C in SGF and SIF as a function of time.

As shown in this figure, the drug release proceeds more efficiently at a higher pH (SIF). The drug-release profiles indicated that the amount of drug released depended on the degree of swelling. The swelling value shows that, an increase in the content of MAA in the feed monomer mixtures resulted in less swelling in SGF but greater swelling in SIF. As the content of MAA in the feed monomers increased, hydrolysis rate decreased at pH 1 but increased at pH 7.4. This was because a higher MAA content in the polymer networks led to higher carboxylate anion concentration at high pH. In other words, the existence of hydrogen-bonding interactions between –COOH groups in the polymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum hydrolyzing of the gel in a medium of pH 1. However,

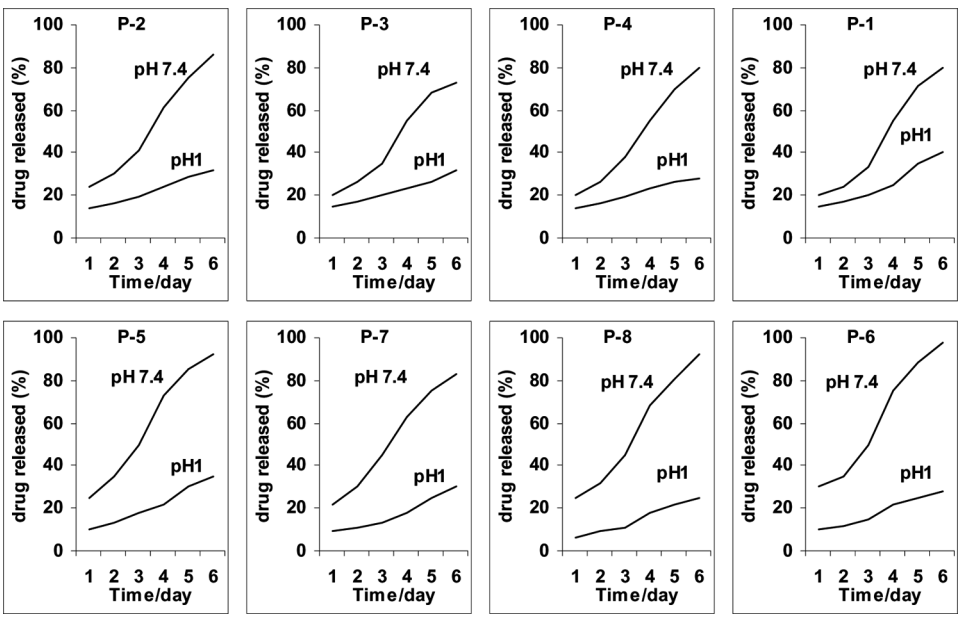


Figure 4: Release of naproxen from hydrogels as a function of time at 37°C.

when the sample is placed in a medium of pH 7.4, the almost complete ionization of $-COOH$ groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged $-COO^-$ groups. These two factors ultimately result in a greater increase in the water uptake. In pH 7.4 with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing agents on the polymer is increased and the hydrolysis rate increased [18]. Therefore, in alkaline pH value, the polymers are easily degraded to release of naproxen.

The presence of grafted PEG chains in these hydrogels plays an important role. At low pH, the oxygen groups in the grafted PEG chain form hydrogen-bonded complexes by interacting with carboxylic groups of PMAA. These hydrogen bonds lead to more collapsed polymer networks resulting in the protection of the drug incorporated in the hydrogels. Moreover, several studies have shown that these grafted PEG chains promote mucoadhesion by chain interpenetration leading to increased drug absorption through the intestinal wall.

CONCLUSION

Novel bioadhesive and pH-responsive hydrogels containing pendent glucose and ibuprofen were synthesized by free-radical crosslinked copolymerization. By regulating the crosslinking percentage of the MAA copolymers, pH-sensitive hydrogels with improved optimal hydrolysis rates were obtained. To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium. The swelling was modulated by the amount of crosslinking of the PBDs prepared. Based on the great difference in hydrolysis rate at pH 1 and 7.4, these carbohydrate polymers appear to be good candidates for colon-specific protein delivery.

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